Colegio de Postgraduados

Optimal growth in juvenile tilapia (*Oreochromis niloticus*), using different levels of fish meal replacement with cricket meal (*Acheta domesticus*)

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ABSTRACT

Objective: To evaluate juvenile tilapia (*O. niloticus*) growth, fed with different inclusion levels of house cricket meal (*A. domesticus*).

Design/methodology/approach: The juveniles (2.16±0.46 g) were placed in 60 L semicircular tanks with 15 fish per tank, and fed at 5% of their biomass in 5 daily rations (08:00; 10:00; 12:00; 14:00, and 16:00 hrs.) for 45 days; temperature (28±2 °C), oxygen concentration (7.2±1 mg/L) and pH (7.02±0.1) were constant. Five treatments were tested: T1-0%, T2-25%, T3-50%, T4-75%, and T5-100%, respectively, with biweekly biometrics. Growth parameters were determined using the biometric data.

Results: Results suggest that AWG, SGR, and PER were higher in T2 but without significant differences with T3; the highest FE was presented in T2 and T3, and the lowest FC, PC, and FCR were found in T3.

Limitations on study/implications: Using insects with sustainable and healthy production can be a viable response to the shortage of fishmeal in aquaculture. However, its use can cause a decrease in palatability and digestibility and a nutritional imbalance in the fish.

Findings/conclusions: Inclusions of 50% showed better growth levels with more vigorous and reactive fish, while higher inclusions affected the growth of *O. niloticus*.

Keywords: Tropical aquaculture, insect meal, alternative fed, food sovereignty, sustainable agriculture.

INTRODUCTION

In Mexico and around the world, the demand for feed for aquatic species has increased in recent years, where the most cultivated species are shrimp, trout, and tilapia; this has led to increased aquaculture production, while production costs are rising due to the shortage of fishmeal due to the decrease in natural fish populations from which it is extracted (FAO, 2020).

Given this situation, new sources of protein have been sought, proposing the use of insects as an alternative source of non-traditional, sustainable, and healthy protein to cover protein requirements in diets that help food security in both humans and animals (Hua, 2021; Maulu *et al.*, 2022; Bingqian *et al.*, 2024). Insect meal has been widely studied in fish diets in recent years. Among the most studied are the house cricket (*Acheta domesticus*), black soldier fly larvae (*Hermetia illucens*), and mealworms (*Tenebrio molitor*). However, it has been observed that the effects are diverse in the different species and are related to the levels of substitution by fishmeal, mainly affecting growth (Guerreiro *et al.*, 2022).

Acheta domesticus meal offers sources of vitamins and minerals with antioxidant properties and a high protein content that can be an alternative source to fish feed (Taufek *et al.*, 2016; Bingqian *et al.*, 2024). However, a nutrient deficiency could affect metabolic pathways and gene expression as a response to a nutritional imbalance, directly affecting growth. The present study aims to know the effects of cricket meal (CM) on the development of tilapia *Oreochromis niloticus*, using different levels of substitution of fish meal (FM) by *A. domesticus* meal.

MATERIALS AND METHODS

Obtaining and maintaining organisms

The Technological Institute of Centla Tabasco, Mexico, donated juvenile tilapia with an average weight of 2.16±0.46 gr. The fish were transferred to the Multidisciplinar Academic Division of Jalpa de Mendez-UJAT (DAMJM), and placed in 80 L semicircular tanks with a density of 15 organisms per tank, a semi-closed recirculation system with two water changes per day, and a temperature maintained at 28±2 °C, with a constant oxygen concentration (7.2±1 mg/L) and pH (7.02±0.1).

Diet formulation and preparation

The *A. domesticus* meal used in this experiment was purchased from the Insect Nutrition company in the Gamm industrial park, Apaseo el Grande, Guanajuato, Mexico. The diet formulation calculations, meticulously performed using the Mixitwin Plus 2.0 program, resulted in isoproteic and isocaloric diets with different levels of fish meal substitution by *A. domesticus* meal. The treatments were T1 control 0%, T2 25%, T3 50%, T4 75%, and T5 100% substitution, respectively (Table 1).

The treatments were prepared in the Academic Division of Biological Sciences, Aquaculture Nutrition Laboratory in the Universidad Juárez Autónoma de Tabasco. For their preparation, the macronutrients (meal used) and micronutrients were passed through a sieve with a 500-micron sieve to obtain the same particle size. The pellets were obtained

Table 1. Formulation of the experimental diets with different levels of inclusion of cricket meal (*A. domesticus*).

Ingredients	0% cricket meal	25% cricket meal	50% cricket meal	75% cricket meal	100% cricket meal
Soft wheat grain	43.44	42.56	42.74	41.86	41.6
Fish meal (herring)	26.01	20	12.83	5.72	0
Soybean meal 44%	20	18.9	18	18	17.03
Cricket meal	0	8	16	24	31
Fish oil	3.05	4	4	4	3.86
Soy lecithin	3	2.05	1.93	1.92	2
Gelatin	2	2	2	2	2
Vit-min premix	1	1	1	1	1
dehydrated fish solubles	1	1	1	1	1
Vitamin C	0.5	0.5	0.5	0.5	0.5
Nutrients	As fed	As fed	As fed	As fed	As fed
MEt Energy(m)	1355.36	1654.59	1987.15	2286.38	2564.29
Protein	35	35	35	35	35
Total fat	10	10	10	10	10
Fiber	2.89	3.54	4.1	4.75	5.27
Ashes	5.07	4.31	3.68	2.92	2.31

as a 4 mm diameter matrix and dried at a constant temperature of around 60 to 70 °C for 10 to 12 hours.

Feeding, parameters measurement and biometric parameters

The fish were fed five daily rations at 08:00, 10:00, 12:00, 14:00, and 16:00 hr for 45 days. Biometry of the weight and size of each organism was performed every 15 days to modify the food rations according to the body biomass of the fish (5%). The organisms were weighed on an analytical balance to calculate the biomass of each experimental unit; the size was measured with an ichthyometer from the head to the caudal fin. The pH reading was performed with a pH meter brand HI98108 pHep$^{+®}$; temperature and oxygen were measured with a YSI 5 oximeter brand Hualix, and the readings were recorded twice a day (8:00 am and 4:00 pm), respectively, to keep the water in optimal conditions.

The biometric parameters were analyzed at the Universidad Popular de La Chontalpa, Absolute Weight Gained (AWG); Specific Growth Rate (SGR); Daily Growth Coefficient (DGC); Protein Consumption (PC); Feed Conversion Factor (FCR): Protein Efficiency Ratio (PER); Daily Weight Gain (DWG); Condition Factor (K) and Feed Efficiency (FE), were determined at the end of the experiment, using the following equations:

$$AWG\left(g / fish\right)=Wf\left(g\right)-Wi\left(g\right)$$

$$SGR(\%)=100\left(\ln Wf-\ln Wi / T\left(days\right)\right)$$

where: *Wi* and *Wf* are the initial and final weight of the fish respectively, and *T* is the number of days in the feeding period.

$$DGC(mg) = Wf^{1/3} - Wi^{1/3} / T(days)$$

$$PC = (Feed\,Consumption(g / day) - (Dietary\,Protein\,Level))/100$$

$$FCR = Dry\,Feed\,Provided(g) / Wet\,Weigth\,Gain(g)$$

$$PER = Wet\,Weigth\,Gain(g) / Protein\,Delivered(g)$$

$$DWG(g / day) = Weigth\,Gain(mg) / Time(days)$$

$$K = Wf(g) / Standard\,Length(cm)^3 \times 100$$

$$FE = Wf(g) - Wi(g) / Feed\,Consumed(g)$$

Statistical Analysis

The data obtained at the end of the experiment were analyzed using the Kruskal-Wallis test. In addition, a Duncan test was used with a significance value of $P<0.05$ for the treatment of means. These statistical analyses were carried out using STATISTICA™ v.7.0 software (Statsoft, Tulsa, USA). Graphics were elaborated using the Sigma Plot 12.0. (Grafiti LLC, Palo Alto, USA).

RESULTS AND DISCUSION

Table 2 shows the results of the nutritional profile of the treatments, which was analyzed in the Aquaculture Nutrition Laboratory of the National Autonomous University of Mexico in Merida, Yucatan. The nutritional profile was observed to be very similar between the treatments, but there is a difference in the percentage of lipids and ash. However, treatment T3 had the most significant similarity with the control, so the proximal content between the commercial feed and the feed made with cricket meal is very similar in nitrogen and carbon content.

At the end of the bioassay, T1 and T2 presented the highest values, showing no significant differences, but concerning T3, T4, and T5, the lowest values for AWG. In SGR, it was observed that T3 showed no significant differences concerning T1, and its difference concerning T2 was slightly significant, with T4 and T5 being where the lowest dietary use was observed (Figure 1a and 1b).

Table 2. Nutritional profile of the experimental diets.

Treatment	Meal (%)	Approximate sample weight sample	Nitrogen (%)	Carbon (%)	Moisture	Ash	Total lipids	Calories/g
T1	0 %	1.074	6.57	45.31	11.460	6.598	3.771	4573.192
T2	25 %	1.049	6.82	46.05	9.703	6.613	6.048	4678.849
T3	50 %	1.032	6.81	45.71	11.796	6.556	4.217	4868.103
T4	75 %	0.949	6.75	46.89	10.079	4.862	7.702	4419.706
T5	100 %	1.118	7.10	46.42	10.129	5.420	5.986	5125.193

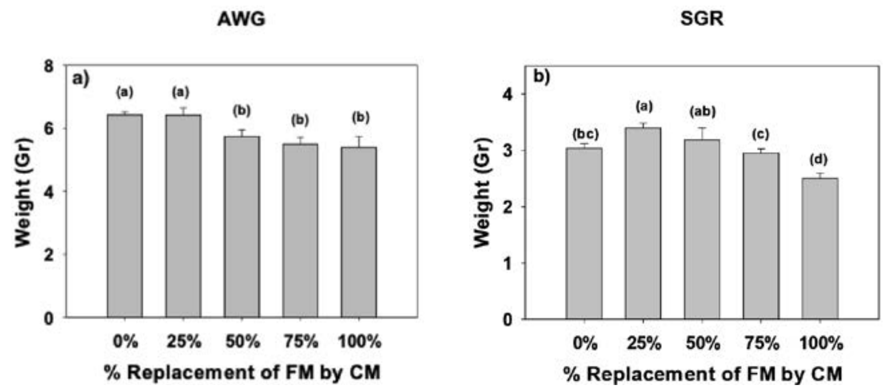

Figure 1. a) Weight gain and b) Specific growth rate of *O. niloticus* with different levels of substitution of fish meal by *A. domesticus* cricket meal.

On the other hand, the lowest FC was obtained in T3, being slightly significant with T2 and T4 and primarily significant with T1 and T5, respectively. Likewise, for the FCR values, T2 and T3 presented the best values concerning T1, T4, and T5, showing important significant differences (Figure 2a and 2b).

Regarding PC, T5 was not statistically different from T1, as T3 revealed the lowest values of crude protein consumed (Figure 3a). Regarding PER, treatments T2 and T3 present the highest values concerning T1, with a slightly significant difference between both treatments. In contrast, T5 presented the lowest protein efficiency, which is statistically different from the rest of the treatments (Figure 3b).

Table 3 shows the growth rate of the DWG in treatment T2 (25%), and it does not show significant differences compared to T1. However, it was significantly reduced in T4 and T5, while the DGC was also affected in treatment T5. Feed efficiency showed a downward effect in T5, and as regards survival, it had no effect in the treatments.

The continued growth of the aquaculture sector has led to an increase in the demand for fishmeal, the primary source of protein in the diet of farmed fish, raising its price and leading to a rise in production costs (FAO, 2020); Therefore, they require efficient and viable alternative protein sources, with studies carried out with plant and animal sources,

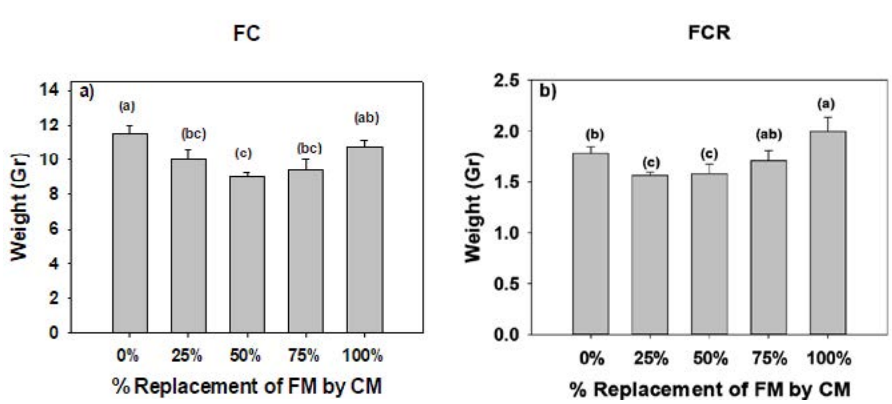

Figure 2. a) Feed consumed and b) Feed conversion rate of *O. niloticus* fed with different levels of replacement of fish meal with *A. domesticus* cricket meal.

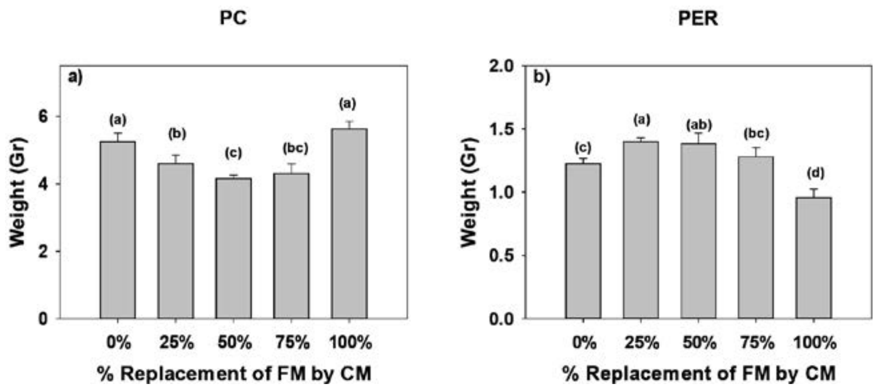

Figure 3. a) Crude protein consumed, and b) the protein efficiency rate of *O. niloticus* fed with different levels of replacement of fish meal with *A. domesticus* cricket meal.

Table 3. Growth of *O. niloticus*, fed with different dietary inclusion levels of cricket meal.

CT	Wi	Wf	LF	DWG	DGC	K	FE	SUP
0%	2.21 ± 0.09^{b}	8.64 ± 0.02^{a}	5.49 ± 0.01^{a}	214.35 ± 3.01^{a}	0.07 ± 0.00^{a}	5.20 ± 0.02^{b}	0.56 ± 0.02^{ab}	100 ± 0.00
25%	1.78 ± 0.08^{c}	8.20 ± 0.25^{ab}	5.40 ± 0.20^{a}	214.12 ± 7.24^{a}	0.07 ± 0.00^{a}	5.22 ± 0.42^{b}	0.64 ± 0.01^{a}	100 ± 0.00
50%	1.80 ± 0.16^{c}	7.54 ± 0.06^{c}	5.13 ± 0.08^{ab}	191.38 ± 7.10^{b}	0.06 ± 0.00^{ab}	5.56 ± 0.24^{b}	0.63 ± 0.04^{a}	100 ± 0.00
75%	1.98 ± 0.09^{bc}	7.48 ± 0.28^{c}	4.59 ± 0.42^{bc}	183.27 ± 7.21^{b}	0.06 ± 0.00^{b}	7.94 ± 2.05^{ab}	0.58 ± 0.03^{ab}	100 ± 0.00
100%	2.59 ± 0.03^{a}	7.97 ± 0.35^{bc}	4.30 ± 0.11^{c}	179.54 ± 11.81^{b}	0.05 ± 0.00^{b}	10.01 ± 1.04^{a}	0.50 ± 0.04^{b}	100 ± 0.00

however, within these sources, insects present a potential source unlike those mentioned above, since they can be produced massively, in less time and space, their production is environmentally friendly (Maulu *et al.*, 2022), it is highly rich in protein (42.1-63.3%), even higher than fish this amino acid profile is highly biodigestible by fish (Alfiko *et al.*, 2022), and its variation between species depends on their nutritional habits, being viable as a food ingredient for several species of omnivorous and/or carnivorous fish in their early stages of life, these dietary amino acids participate in the regulation of blood biochemical parameters and the gastrointestinal microbiota (Mikołajczak *et al.*, 2020), as well as modifying the gene expression of fish digestive enzymes, which in turn can affect digestibility and growth (López-Cerino, 2023).

In our study, juvenile *O. niloticus* showed a higher growth performance and SGR in the initial stage of the production cycle and lower in the later stages, like what was reported in Rohu (*Labeo rohita*) and *O. niloticus* by Rahman *et al.* (2021). Although there are studies that reveal a reduction in fish growth when more than 30% of FM is replaced by insect meals (Hua, 2021), the SGR in the present investigation showed that the inclusion of 50% cricket meal may be viable for the growth of tilapias, since it did not show significant differences compared to the control treatment. Likewise, a correlation was observed between a higher FCR value and a lower SGR value, previously reported in *O. niloticus* by Rahman *et al.* (2021); this is because the FCR is the relationship between the food consumed by the fish and the body weight gain obtained, while SGR expresses growth as the intuitively understandable percentage change in size per unit of time. In perch (*Perca* *fluviatilis*), replacing 25% of the FM with a mixture of *A. domesticus* found a decrease in growth and an increase in FCR (Tilami *et al.*, 2020). It should be noted that not all fish have the same FCR, and this is because the utilization and use of nutrients vary among species. In our study, the lowest feed consumption and the highest FCR were found in T3, while the highest feed consumption and highest FCR were found in T5; the latter may be due to a nutritional deficiency that increased the intake of the fish as a mechanism to satisfy their needs (Concha-Frías *et al.*, 2018); this is because feed consumption is strongly linked to the amount of protein in the diet and modifies the satiety of the fish, something observed in our study. The same behaviors were reported by Nadaf *et al.* (2010), where they observed that the higher the FCR, the higher the SGR in juveniles of *L. rohita*. Likewise, weight gain, SGR, and FCR are significantly affected by the feeding rate, so sometimes a more significant amount of feed is required to increase growth as observed in the rabbitfish (*Siganus guttatus*), which will depend on the culture method according to Heriansah *et al.* (2022).

Regarding growth performance, such as weight gain and specific growth rate, these increased significantly in diets with a cricket meal level of up to 50% and then decreased with increasing cricket meal levels of up to 100%. Cadena-Cadena *et al.* (2023) report report that the inclusion of 35% of *A. domesticus* in *O. niloticus* juveniles did not affect growth; likewise, in totoaba juveniles (*Totoaba macdonaldi*), Carvajal-Soriano (2022) reports similar data, recommending that substitution levels of up to 50% of fishmeal with *A. domesticus* meal are feasible without compromising survival or growth, while Lee *et al.* (2017) report that at optimal concentrations of cricket meal (60% *A. domesticus* and 40% rice bran) good growth occurs in *Oreochromis* sp, but at higher concentrations, they present liver damage.

In this study, PER was higher, and FCR was lower in fish-fed diets containing 50% inclusion than in the control. Hua (2021) has recommended these low values of insect meal replacement accepted by fish. They are due to the nutritional value of the meals since they present deficiencies in some amino acids and fatty acids essential for the physiology of fish, as well as high levels of chitin that decrease palatability and affect some metabolic and physiological pathways (Roncarati *et al.*, 2015).

However, it has been observed that 100% replacement of fishmeal with *A. domesticus* meal and field cricket (*Gryllus bimaculatus*) meal increases SGR, DWG, and AWG in *O. niloticus* fingerlings (Perera *et al.*, 2023). In Guppy fish (*Poecilia reticulata*), 100% replacement of fishmeal with *A. domesticus* and/or *G. bimaculatus* meal did not affect growth, noting that field cricket meal improved fish pigmentation (Perera & Bhujel, 2022).

Regarding the utilization of cricket meal in the study, T3 presented the lowest value and a higher PER, indicating a better utilization of the diet by the fish. Similar data are reported by Bhilave *et al.* (2012) for *C. idella* fed with diets formulated with soybean meal, highlighting that reducing the dietary protein level without affecting growth can reduce feed cost. Likewise, in mahseer (*Tor putitora*), an optimal protein requirement of 45% is reported, where the SGR and PER were higher with the lowest FCR (Ullah *et al.*, 2022), while Concha-Frías *et al.* (2018) report that a higher PER goes hand in hand with a lower CP, as well as a higher SGR and AWG in the common snook *Centropomus undecimalis*. It is that an excess of amino acids consumed by the fish must be eliminated from its system, causing an extra energy expenditure in which amino acids are used for energy production (ATP) since the fish cannot accumulate these surpluses, thus reducing growth, due to an increase in the catabolism of lipids and carbohydrates in the same process (Concha-Frías *et al.*, 2018), this leads to an increase in feed consumption and little use of protein, similar to what was observed in our study where PER and FC are higher in T4 and T5 respectively.

Optimal replacement of insect meal in fish has shown a decrease in oxidative stress, which may be related to its chitin content and lipid fraction since the latter are more susceptible to oxidation (Guerreiro *et al.*, 2022; Bingqian *et al.*, 2024). However, in most studies, dietary inclusion of 50% and even 75% can present a significant decrease in the growth rate and a decrease in the feed efficiency of the fish, increasing oxidative stress (Bingqian *et al.*, 2024), which may be due to the deficiency of some essential amino acids in the tilapia diet, which could have an impact on the growth and health of the fish (Gasco *et al.*, 2016; Guzmán-García, 2023), as observed in our bioassay.

High inclusions of cricket meal (T4 and T5, respectively), showed a low growth of tilapia, which may be related to high protein digestion and, therefore a high energy cost for the elimination of excess amino acids (AAs) (Concha-Frias *et al.*, 2018), it may also be due to a deficiency in essential and/or limiting AAs in the diet, such as arginine, methionine, leucine, and lysine (Guerreiro *et al.*, 2020; Alfiko *et al.*, 2022), since the deficiency of these amino acids can cause a decrease in the production of various metabolites such as nitric oxide (NO), polyamines, urea, proline, and glutamate (Ahmad *et al.*, 2021), as well as a deterioration in defenses, protein synthesis, DNA methylations, decreased functions of antioxidants, metabolizers, immune system, disease resistance, cell signaling and endocrine system, cause DNA damage and therefore trigger cell apoptosis, cancer and even death (Yen *et al.*, 2002; Bingqian *et al.*, 2024), so high levels of inclusion of these meals could increase oxidative stress levels and decrease fish defenses (Taufek *et al.*, 2016; Maulu *et al.*, 2022). Guzman-García (2023) indicates that the increase in the concentration of cricket meal in the diet of the Mojarra Zacatera (*Cincelichthys pearsei*) caused an increase in the gene expression of BCL2 and BAX, P53, and Caspase involved in cellular DNA repair and apoptosis, which may be a reflection of the damage caused by the release of reactive oxygen species, due to oxidative stress caused by the diet, affecting the activity of antioxidant enzymes such as catalase and superoxide dismutase, etc., thus generating severe metabolic damage to proteins and lipids which is reflected in a decrease in fish growth (Song *et al.*, 2023).

Another nutritional component linked to poor growth in fish-fed insect meals is chitin, which is usually not digestible for many fish species and usually decreases the diet's palatability in large quantities. Some fish can digest chitin because they have enzymes such as chitinases, thus improving digestibility and facilitating the work of other digestive enzymes; they may also have the enzyme chitobiase in the intestine, which hydrolyzes chitin into β N-acetyl-glucosamine monomers to be absorbed by the enterocytes of the intestinal wall according to Gutowska *et al.* (2004). However, in fish that do not possess enzymes that allow the degradation of chitin, they increase the production of hepatocytes capable of managing metabolic needs; therefore, high concentrations of this carbohydrate decrease the palatability and digestibility of the diet, affecting the gene expression levels of proteolytic enzymes (Carvajal-Soriano, 2022; López-Cerino, 2023), decreasing the activity of trypsin and causing low protein hydrolysis (López-Cerino, 2023). Likewise, organisms obtain most of their energy from lipids, which are part of the composition of the membrane, cell signaling, regulation of oxidative stress, and nutrients (Yoon *et al*., 2021). Fish oil contains a higher concentration of omega-3 fatty acids than insect meals, which have a higher concentration of saturated fatty acids (linolenic acid and n-6 fatty acids), which is favorable for the health of fish consumers (Lee *et al*., 2017; Tilami *et al*., 2020) and low content of LC-PUFAs, while the concentrations of unsaturated fatty acids are higher in the domestic cricket (around 60-70%) (Gasco *et al*., 2016; Alfiko *et al*., 2022). An imbalance in the concentration of fatty acids required by the fish affects its health (Guerreiro *et al*., 2020), activating oxidative stress through lipid peroxidation (PUFA oxidation), reducing antioxidant protection and fish growth.

CONCLUSIONS

The protein content of the T3 treatment based on 50% cricket meal did not show significant differences compared to the control treatment, which did not contain cricket meal. Regarding the deficiencies presented by cricket meal, these were supplemented with a premix of vitamins and minerals, such as soy lecithin, since these inputs are essential for the growth of tilapia. The productive parameters were not affected by the levels of substitution applied, with T3 (50%) being the most similar to the control treatment because the percentage of protein is very similar between these treatments. However, in higher concentrations, they affect growth in a negative way, which may be strongly related to an amino acid deficiency, lipid deficiency, and an excess of chitin. Using the house cricket to produce meals as feed for the cultivation of tilapia in the early stages of fry is feasible because it is rich in protein and has a simple production process with no ecological impact.

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